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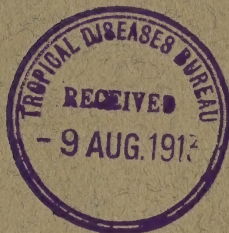
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NOTES ON THE ETIOLOGY OF RELAPSE *W. M. James*
IN MALARIAL INFECTIONS

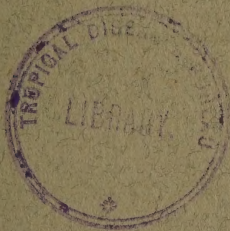
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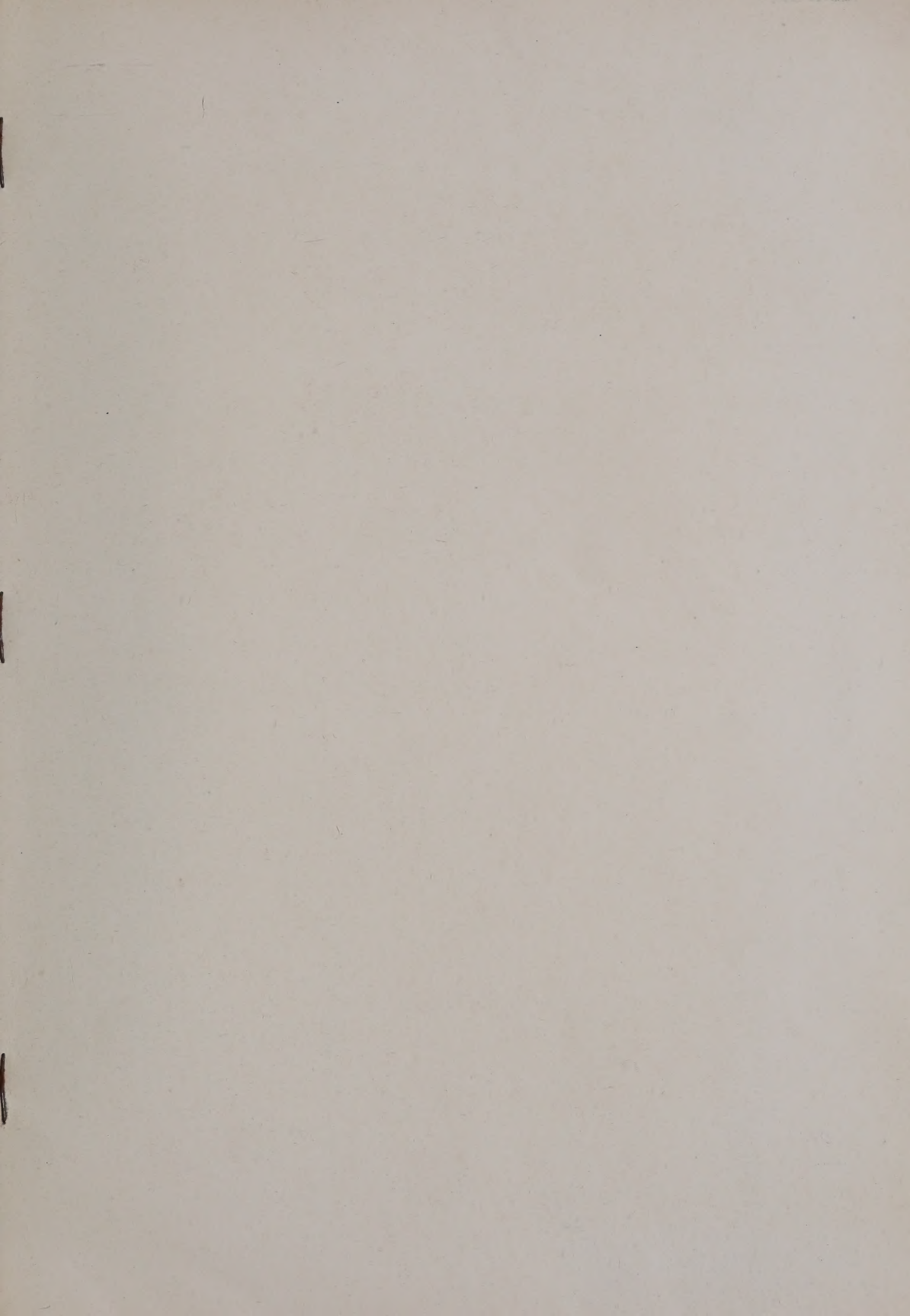


PLATE I.



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NOTES ON THE ETIOLOGY OF RELAPSE IN MALARIAL INFECTIONS.*

W. M. JAMES.

(From the Ancon Hospital, Ancon, Canal Zone.)

(WITH PLATE 1).

I.

In a recent article Dr. Graham A. Henson[†] drew the attention of physicians in the southern states to the fact that while our knowledge of the transmission of malaria, the morphology of the parasites, and the methods of treatment and prophylaxis may be considered fairly complete, the important subject of the etiology of relapse is far from determined. In a general way Dr. Henson's statement is correct; but in spite of our best methods, relapses occur after all systems of quinine administration, showing that today our treatment is not seldom at fault. It may be stated also that a definite knowledge of the morphology of malarial parasites is not any too widespread, for in following closely recent literature, one often finds that descriptions of what are called "malarial parasites,"

* Received for publication March 3, 1913.

[†] *South. Med. Jour.*, 1912, 5, p. 450.

or peculiar and rarely found forms of these, are such as to cast suspicion on the accuracy and correct interpretation of the observations. There are not a few who denounce many of the methods of quinine prophylaxis as worse than useless, often apparently with very good reason.

However, it is an undisputed fact that relapse in malaria is responsible for many of the evils that follow infection with one or more species of *Plasmodia*. There is some reason to believe that primary attacks, even with estivo-autumnal infections, are not as fatal as are relapses or their equivalents, repeated reinfections. In the Canal Zone often we cannot say with certainty whether any particular subsequent attack is a relapse or a reinfection; since most of us live and work in the same place, it follows that if the place can harbor the source of original infection, reinfections will surely follow if the cause is not removed.

Notwithstanding the difficulty of stating definitely the actual proportion of relapse, I do not hesitate to affirm that no inconsiderable part of the malaria here prevalent is due directly or indirectly to it. For the present by a relapse I mean "a return of the fever and parasites after the former has ceased and the latter have disappeared from the peripheral blood, reinfection being excluded. I do not include those cases in which quinine has not been administered in doses sufficient to clear the peripheral blood of parasites. Such cases are not true relapses, for the original infections are only diminished."¹

To prove that a large part of malaria here (and elsewhere as well) is due to relapse, I invite attention to the following data.

1. In certain places, for example, sections of Italy and Greece, and the northern part of the southern states, where a great quantity of malarial infection is present during the summer and autumn, in the winter, when the possibility of reinfection is slight or absent, there is nevertheless a very considerable amount of the disease. This shows that when large numbers of people are infected in any community, a certain proportion, after the acute symptoms have subsided, will carry the parasites in some latent manner until a favorable opportunity supervenes for the necessary increase to produce fever. In temperate regions possibly undue exposure to cold or some other debilitating cause creates this opportunity. Here in the tropics, exposure to rain with subsequent chilling, as I shall presently show, or lowered resistance due to other disease, or insufficient food, very likely act similarly. These statements are qualified because

¹ *Proc. Canal Zone Med. Soc.*, 1910, 3, p. 29.

until the entire etiology of relapse is definitely proved, I do not care to set forth as fact what depends on circumstantial evidence, however well founded.

2. It is not uncommon to witness a relapse of malaria, in the wards of this hospital, during the course of another disease, but that it occurs more often after convalescence is well established. If, as in the latter instance, a relapse takes place while the patient is resting, surely it is not unreasonable to suppose that relapses occur in greater numbers among those whose resistance is lowered by exposure and hard labor.

3. In the Canal Zone, it has been shown elsewhere¹ that the increase in the amount of malaria at the beginning of the wet season is out of proportion to the increase in the number of Anopheles mosquitoes. It takes time for mosquitoes to breed, to become infected themselves, and so to transmit infection; but the malaria rate rises rapidly and promptly immediately after the first heavy rains, even in Zone towns in which the mosquito rate shows no perceptible increase. Although the rains may drive the Anopheles indoors, thus adding to chances of infection or reinfection, still this increased number of mosquitoes indoors is not sufficient to account for the increased malaria rate. Further, at the close of the wet season, the malaria rate falls promptly with the cessation of the rains, and much more rapidly than does the mosquito rate. These facts show a very circumstantial relation between exposure to wet and chilling and the increase in the malaria rate, and between the cessation of this exposure and the decrease in the malaria rate.

4. Insufficiently treated infection with any species of *Plasmodia* is exceedingly prone to relapse, however apparent the temporary cure may have been. In the opinion of those whose experience enables them to speak with authority, quartan infection, though well treated, relapses more frequently than does infection with estivo-autumnal or tertian parasites. As far as my limited experience with quartan infection goes, the opinion is correct.² But the number of quartan infections in the Canal Zone is too small to permit of accurate deductions. Still, it is a matter of common knowledge here that estivo-autumnal infections are as a rule much more difficult to eradicate, and require much more extended treatment to effect a cure than tertian infections, although some tertian infections are very resistant to treatment. I have recently seen a patient who had five relapses with tertian malaria in three months, the last after an intravenous injection of 22.5 grains of quinine, and quinine by mouth for 10 days, in doses of 10 grains three times a day.

As a rule, owing to local conditions, estivo-autumnal and tertian infections among the Negroes and European laborers receive the same treatment and for practically the same time, the average stay in hospital being about a day longer for estivo-autumnal infections. If primary infection or reinfection were responsible for most of our malaria at the beginning of the wet season, tertian malaria should increase proportionately with estivo-autumnal, because Dr. S. T. Darling³ has demonstrated that *A. albimanus*, the most common malaria carrier here, is as susceptible to infection with the one species as with the other. It has also been shown that while the gametes of *P. falciparum* (the estivo-autumnal parasite) persist longer in the peripheral circulation than do those of *P. vivax* (the tertian parasite), the latter occur here relatively more often. I find gametes in nearly all tertian infections, and crescents in amounts sufficient to infect mosquitoes in a small proportion only of estivo-autumnal infections. If the increase in the malaria rate at the beginning of the wet season were due

¹ Deeks, W. E., and James, W. M., *A Report on Hemoglobinuric Fever in the Canal Zone*, 1911, p. 19.

² *Proc. Canal Zone Med. Soc.*, 1910, 3, p. 29.

³ *Ann. Trop. Med. and Parasit.*, 1910, 4, p. 179.

solely to primary or subsequent infections, these facts show that tertian malaria should, as has been noted, increase proportionately with estivo-autumnal. But the contrary is true, for in European laborers, who are very susceptible to malaria, and in Negroes, who are comparatively immune, the estivo-autumnal rate at this time rises, relatively as well as proportionately, greatly when compared with the tertian rate. Particularly is this true among the European laborers.¹

Without relapses, then, the malaria rate should rise synchronously with the increase in the mosquito rate; but with relapses, if these produce gametes in the same proportion as primary infections or reinfections, as much evidence seems to indicate, the malaria rate is augmented not only by the number of cases due to relapse, but by the increase later in primary and subsequent infections due to the greater quantity of infection in mosquitoes, though the total number of mosquitoes may remain the same for some time.

These data will, I trust, convince of the importance of relapse as a factor in the prevalence of malaria here and elsewhere. Relapses are of importance to the sanitarian because, as Dr. Henson points out, if all primary cases were cured so that gametes could no longer be produced, and relapses with gamete production were prevented, malaria would thereby be eradicated. Relapses are of importance to the physician, since what is called "chronic malaria" results as much, if not more, from them as from reinfection. Further, a relapse means insufficient treatment of the primary infection, and those who are content to effect a temporary cure without regard to future evil would do well to remember this. One might as well treat syphilis with one or two doses of mercury, as to treat a malarial infection with five grains of quinine three times a day for a week and expect a cure.

II.

In September, 1911, the Department of Sanitation of the Isthmian Canal Commission sent out to various authorities on malaria throughout the world a circular letter, in which information was requested as to the amount of malaria due to relapse, the various predisposing causes, the prophylaxis of relapse, and the etiology and subsequent effects. The purpose was to obtain data upon which a research could be made with the object of excluding or verifying various hypotheses current at the present time as to the etiology of relapse, and the best methods of prophylaxis and treat-

¹ Deeks, W. E., and James, W. M., *A Report on Hemoglobinuric Fever in the Canal Zone*, 1911, p. 26.

ment against it. Nearly all replied to these letters, and the information thus collated is now being used in an effort to obtain definite conclusions. As it is impossible at this time to present a full account of the work done in the past year, I shall limit myself in this paper to a discussion of the various hypotheses as to the etiology of relapse, and the results of my work during the last 12 months in studying anew this question in the light of the data which has so generously been furnished me.

Until the true reason for the cause of relapse is known, the treatment of malaria will remain somewhat empirical, for practically all of the authorities referred to above agree that a very large proportion of malaria, from 50 per cent to 90 per cent in malarious communities, is due to relapse; and further, as stated in the introduction, such relapse is very largely responsible for the continued propagation of malaria. Though in varying proportion, relapses have followed every method of giving quinine, from prompt and vigorous treatment of the original attack by large doses of quinine administered intravenously, to small doses in quantity just sufficient to control the fever, and no more. This means either that quinine as exhibited today is not administered properly, or that the drug is not the specific that most textbooks hold it to be, in so far as it effects a permanent cure. I do not mean that quinine is a failure, or that I would advocate the use of another drug. I wish only to emphasize the fact that as long as relapses are responsible for the present proportion of attacks of malaria, the proper treatment has not been effected. Undoubtedly the fault is by no means altogether with the drug; perhaps the perfect method of administration has not yet been found. When it is, there will be no more malaria in properly treated communities, as Dr. Henson has noted. In the meantime, the determination of the true cause of relapse is a matter of highest importance, for it is beyond all question that this cause is intimately concerned with some stage in the life-cycle of the *Plasmodia* over which quinine has little or no control.

It is my purpose at present to advance certain propositions as to some factors common to relapse, that are derived from the extended clinical observations and experience of the authorities

referred to above; and to apply the data deduced from these latter to the various hypotheses that have been advanced as to the etiology of relapse. I shall add, also, the opinions which I hold at present as a result of my work on this subject.

1. Today, as in the past, relapse is a very common feature of malarial infection.
2. Relapse almost invariably follows the so-called spontaneous cure of primary cases of malaria; that is, the cessation of symptoms without treatment.
3. Infections treated insufficiently with small doses of quinine will in all probability relapse.
4. The sooner prompt and vigorous treatment is instituted after the onset of the primary attack, the less likely is relapse to follow; and conversely, the later the primary attack is treated, even with large doses of quinine and for a long time, the more certainly will the symptoms recur.
5. Sometimes a relapse, with parasites in the peripheral blood, will take place although the patient has not stopped taking quinine. These cases are rare, but I have noted several in Ancon Hospital.
6. If reinfection is excluded, and death does not take place during a relapse, in time the infection will die out. Otherwise there would be no immunity, and every untreated case would end in death. This applies in the last analysis even to persons who are "carriers," and who manifest no febrile symptoms, although parasites may be found in the peripheral blood.
7. It is easier to effect a permanent cure for persons in good health prior to the attack, than for those in whom the malarial infection is complicated by bodily weakness or intercurrent disease, especially syphilis.

The hypotheses as to the cause of relapse fall under two heads:

1. Some particular, or peculiar, form of the parasite is developed during the asexual cycle, and this form is resistant to quinine and to the protective forces of the body. It remains latent until called into activity by certain forces whose action is not as yet clearly understood, when it breaks up into merozoite-like parasites, which take up anew the asexual cycle.

This is the hypothesis which explains relapses either as due to parthenogenesis of the female gamete, or macrogamete, as advanced by Cannalis,¹ Grassi,² Maurer,³ Neeb,⁴ and others, and worked out in its greatest detail by Schaudinn;⁵ or as due to segmentation of a parasite formed by conjugation of two young asexual forms, which is the view taken by Craig.⁶ Mannaberg⁷ believes that

¹ Cited by Deaderick, and by Marchiafava and Bignami.

² *Ibid.*

³ *Centralbl. f. Bakteriol.*, Orig., 1902, 32, p. 695.

⁵ *Arch. a. d. k. Gesandheitsamte*, 1903, 19, p. 169.

⁴ *Jour. Trop. Med.*, 1910, 13, p. 98.

⁶ *Jour. Infect. Dis.*, 1910, 7, p. 285.

⁷ "Malarial Diseases," Nothnagel's *Encyclopedia of Practical Medicine*, Philadelphia, 1905, p. 52.

crenations are formed by the union of two or more of these young forms, but I can find no statement of his which would indicate that he upholds the parthenogenetic hypothesis;¹ while Ewing,² who also observed conjugation, states: "The large size of the sporulating forms developed from conjugating pairs suggests that this process is intended to especially favor the multiplication of the species in the human host." Ewing's observations were made, as were those of Schaudinn, on the tertian parasite, while those of Craig were made on all three species.³

2. After a diminution in their vitality, due either to quinine or to the natural protective forces of the body, the asexual parasites continue to go through their normal life-cycle, but in very small numbers (relatively speaking), until the causes that bring about relapse effect a renewal of vitality, so that the parasites increase in number until the clinical symptoms of relapse are manifested.

This hypothesis has the support of Ross and Thomson,⁴ and, slightly modified, of Bignami⁵ also. The last believes that the parasites in their asexual cycle may become immune, either to the natural protective forces of the body, or to the action of quinine.

These hypotheses have two factors in common: the renewal of the asexual cycle to a degree when clinical symptoms are manifested; and a resistance of the latent parasites, whatever may be their form, to quinine.

In discussing these hypotheses I shall refer only briefly to the *a priori* reasons in favor of them, or to the contrary. These are discussed at length by Ross, Craig, Deaderick,⁶ Bignami, and Henson. It is my purpose now to examine these hypotheses in the light of the propositions above mentioned, and to bring out certain facts derived from my own observations and those of others.

¹ Ross, in the *Prevention of Malaria* (p. 110), quotes Mannaberg as stating, in 1894, that relapses in localities free from malaria are probably due to "crescentic bodies."

² *Clinical Pathology of the Blood*, New York, 1903, p. 454.

³ Craig states that Ewing also observed conjugation in the estivo-autumnal species.

⁴ *Ann. Trop. Med. and Parasit.*, 1910, 4, p. 137.

⁵ *Sulla Patogenesi delle Recidive nelle Febbri Malariche, Estratti degli Atti per gli Studi della Malaria*, Rome, 1910, Vol. 11. Translated in *South. Med. Jour.*, 1913, 6, p. 79.

⁶ *Practical Study of Malaria*, Philadelphia, 1910, p. 124. Also "Recurrences in Malaria, Their Cause and Prevention," *Bull. Soc. Path. Exot. Par.*, 1910, 3, p. 498.

THE FIRST HYPOTHESIS.

Although observers had claimed parthenogenesis as a cause of relapse prior to Schaudinn's publication, he was the first to see the process in a species of malarial parasite other than the estivo-autumnal, and to describe it minutely. In the examination of the blood of a patient who had at the time a relapse of tertian malaria, he found certain changes in the macrogametes which he interpreted as parthenogenesis, or reproduction by the female parasite without fertilization. Blüml and Merz,¹ and Karrewij,² later reported findings in tertian infections similar to those of Schaudinn, and Neeb, and Maurer, described sporulation of the female crescent. Later Major W. S. Harrison³ published an account of parasites found by him in a case of tertian infection, which seemed to correspond with those described by Schaudinn as parthenogenetic forms, and gave very excellent illustrations. Major Harrison was reserved in his interpretation of these forms, which he found in two cases, one of relapsing malaria, and the other very probably from a relapsing case. As far as I am aware, no confirmation of parthenogenesis has been obtained accurately in quartan malaria, notwithstanding the well known tendency of this type to relapse.

The essential feature in all descriptions of the process, including and following that of Schaudinn, is the division of the chromatin into two parts. One of these, the *Restkörper*, degenerates, or at least has nothing further to do with segmentation, while the other undergoes amitotic division and forms the nuclei of merozoite forms, as in asexual division.

Craig claims that in all three forms of malarial infection there is, when conditions are favorable, a conjugation of two young asexual organisms within the same erythrocyte, with subsequent union of nuclei and cytoplasm. From this union results a parasite which grows rapidly, differs in appearance from the organisms of the sexual and asexual generations, and is resistant to quinine. Craig has not followed these organisms from conjugation to complete sporulation but has witnessed all stages, including the actual

¹ Cited by Deaderick, *Arch. f. Schiffs- u. Trop. Hyg.*, 1908, 12, p. 249, and by Bignami, from *Geneeskundig Tijdschrift voor Nederlandsch-Indië*, 1908.

² Cited by Bignami and by Neeb.

³ *Jour. Roy. Army. Med. Corps. Lond.*, 1909, 13, p. 647.

conjugation of the living organisms, to the full grown forms, which latter he interprets as the latent organisms. Craig¹ states:

"These developing parasites are distinguished from other forms of the plasmodia by the collection of the chromatin in two or more irregular deeply staining masses distributed about the center of the organisms. In some instances the chromatin masses are numerous and are collected at one side of the organism, giving rise to an appearance suggesting sporulation. Such forms are very suggestive of the parthenogenetic organisms pictured by Schaudinn. [Craig notes that his older forms are very rare in the peripheral blood.] . . . They are certainly not macrogametes undergoing parthenogenesis, as they occur only when conjugation is present, and very frequently before gametes have appeared in the blood. In estivo-autumnal infections these large pigmented forms are only observed in the blood obtained by splenic puncture, and they are not observed in any form of malarial infection in which intracorpuseular conjugation is absent."

Craig adds as his belief that some of these bodies are the same as those considered by Schaudinn to be macrogametes undergoing parthenogenesis; and Deaderick, who upholds the parthenogenetic hypothesis, states that while the forms observed by Craig may result from conjugation, "there is as yet no positive evidence that the latter are not macrogametes," a partial acceptance of Manna-berg's hypothesis as to the formation of gametes.

From the above statements it is very likely that some of the same forms of parasite have been observed both by Craig and by Schaudinn; the correct interpretation of their origin, function, and ultimate fate is the point in doubt.

The points in favor of Schaudinn's hypothesis may be summarized as follows:

1. The persistence of the gametes in the peripheral blood after the disappearance of the asexual parasites. Especially in tertian and quartan infections do the macrogametes persist. It is the consensus of opinion, and my own observations tend to confirm this; that in estivo-autumnal infections both microgametocytes and macrogametes persist. Since gametes of all species are believed to be notoriously resistant to quinine, it is thought probable that they alone survive to renew the asexual cycle.

2. Schaudinn's hypothesis also partly fits the propositions that I have previously advanced, because:

- a) Gametes are common features of untreated, improperly treated, and protracted malarial infections.

- b) They occur most frequently in these.

- c) They do not, however, occur at the onset of the clinical symptoms in primary attacks. But in tertian infections they occur earlier than in estivo-autumnal and quartan primary attacks. And, as noted, it is the promptly treated primary infections that are less likely to relapse.

¹ *Op. cit.*, p. 309.

None the less, despite this favorable evidence, I do not think that further observations, and the opinion of those who have studied the blood in many infections and over a period of some years, will justify an acceptance of Schaudinn's views. Bignami objects to Schaudinn's hypothesis on two points: he does not believe that the observations of those who claim to have confirmed Schaudinn's hypothesis were well founded, and he criticizes the work of Schaudinn himself as follows:

"But it is a fact that only once, and in one patient only, at the beginning of a relapse, the whole series of forms occurred. In spite of this he [Schaudinn], after he had given a most accurate account in his report, expressed the *conviction* that relapses after long intervals owe their origin to macrogametes, which live a long time, and have the capacity, according to him, of undergoing a reversion to the form of schizonts.

"Although the work of Schaudinn was put out in 1903,¹ the process that he described has not received even the most infrequent confirmation by new observations.² The unconfirmed opinion based on that report has been listened to with favor by some, as though it were a datum of fact at this time unassailable."³

Further, Bignami cites evidence to show that the supposed confirmation of Schaudinn's work rests upon unconnected and isolated observations. In most cases when the supposed parthenogenesis occurred, sporulation or other asexual forms were also observed either at the same time, or shortly before. This is true, even of Schaudinn's observation, as a careful reading of his paper in the light of subsequent knowledge will demonstrate that these were by no means excluded.

I believe, as a result of my observations in the past few years, that Bignami's objections are very well founded. It is well known that when the environment is unfavorable, any species of protozoon will take on atypical developmental and reproductive forms. Craig⁴ has accurately described the changes due to quinine in the malarial parasites, and I have noted that the same changes are to be seen in the organisms in untreated infections, when the parasites are lessening in number. Particularly is this true of the segmenting and presegmenting forms. In these, very often the entire chromatin is not used as nuclei for the succeeding generation, but sometimes as much as half of it remains undivided. Such parasites,

¹ 1902 is the correct date.

² That is, not that the forms as described do not occur, but there is no evidence to prove that they are responsible for relapse.—W.M.J.

³ *Op. cit.*

⁴ *Loc. cit.*

however, do not attain the size pictured by Schaudinn for his parthenogenetic forms, which fill the entire swollen erythrocyte. But in anemic blood, such as is found in persons who are suffering from relapse (when the infection is heavy so that many forms may be observed), the environment is also unfavorable for the development of a typical life-cycle, because the erythrocytes themselves do not contain enough nourishment for the rapidly developing parasites. It is well known that the parasites depend on the hemoglobin content of the erythrocytes, which they transform to pigment, for food. The occurrence of these very heavy infections in decidedly anemic persons has not been common in my experience (relatively speaking), but I have witnessed such infections several times, and with all three species of parasites. In the blood of these patients are found very large parasites, which show marked vagaries in development, especially in tertian and quartan infections. In estivo-autumnal infections, the changes are slight, if any. These forms correspond in every way to those pictured by Schaudinn, but I cannot interpret them as sporulating macrogametes, because every phase of transition between these and normal segmenting parasites may be observed, whereas the macrogametes very early take on and keep until maturity a distinct developmental cycle. Moreover, my slides have been examined by Dr. Fülleborn, of the Hamburg School of Tropical Medicine, and he informed me that the forms just described are identical with those described by Schaudinn for parthenogenetic forms of the macrogamete. Dr. Fülleborn had seen Schaudinn's original slides, and had observed similar forms of parasites in other specimens. He was also of the opinion that the forms in question were probably atypical developing parasites. In a quartan infection that had persisted untreated for six weeks, in which the anemia was profound, as to number and hemoglobin content of the erythrocytes, I observed parasites of extraordinary shape and size, some 10 m. in diameter, and with most remarkable phases of chromatin division in the nuclei of the adult parasites. But between these and normal quartan parasites was every transitional phase.

It may be objected that if there is not sufficient nutriment the parasites could not attain so large a size. But Calkins¹ has shown

¹ *Protozoology*, New York, 1909, p. 102.

for *Paramecium*, in unfavorable circumstances, that while many of the forms were stunted, as was also the case in the malarial infections to which I have just referred, some organisms, in which division was not complete by reason of lack of vitality to finish it, attained a size out of all proportion to the normal cycle. I have observed a similar phenomenon in *Entamoebae* in which two nuclei were plainly visible without division of the cytoplasm. It does not follow that the cytoplasm loses its power of growth simultaneously with that of the nuclear material. As I have stated, such parasites are not often found in malarial infections, and perhaps because of this rarity, they are interpreted as explaining obscure phases of the life-cycle. The same mistake has been made, and very likely will continue to be made, in the interpretation of seldom found forms in other pathogenic protozoa.

It has been noted that Schaudinn's hypothesis has not received any definite confirmation. With the exception of Major Harrison's report, referred to above, I can find no writers, other than those quoted by Deaderick and Bignami, who claim to have witnessed the process. Deaderick¹ says:

"Golgi plainly stated it as his belief that the crescent was the parasite of fevers recurring at long intervals.

"Canalis, in 1889, described and pictured spherical bodies derived from crescents in the act of sporulation. In 1890, Antoseli and Angelini confirmed the observation of Canalis. Lewkowicz reported, in 1897, that he had seen sporulating crescents some of which contained as many as 30 spores.

"Grassi expressed the opinion in 1901 that the parasites of malaria underwent a parthenogenetic cycle whereby the species was perpetuated after the death of the schizonts.

"Maurer, in 1902, observed a sporulation of estivo-autumnal gametes, and construed it as parthenogenesis.

"Ziemann believes that he has seen parthenogenetic reproduction of quartan gametes.

"Blüml and Metz observed sporulating parthenogametes in six preparations taken from five patients with tertian malaria. The process was identical with that described by Schaudinn. Young and sporulating schizonts and young gametes were present in these same preparations."

In addition, are the observations of Karrewij, in tertian malaria, cited by Bignami, and of Neeb, which latter I have examined.

Much of this literature is inaccessible to me, but I can cite

¹ *Op. cit.*

opinions of competent authorities who have given it careful consideration.

Marchiafava and Bignami state:¹

"Grassi and Feletti claim to have seen two forms of segmentation of the crescents, one of fission scarcely begun, the other of completed fission, similar to what is seen in the parasites of the regular fevers. Canalis describes the sporulation of round bodies of semilunar origin, and even gives a drawing of it. Golgi held that in the crescents there was a 'process of internal differentiation,' which led to the emission of young parasites which invade new red corpuscles, whence occur renewed febrile attacks. . . .

"Those who have held that there was a multiplication of the crescent bodies without being able to demonstrate it, based their belief upon the fact that the crescents persist in the blood during the apyretic interval separating a group of febrile paroxysms from the relapse, and thought that the latter could be explained only by a process of sporulation of the crescents themselves. Those who have described and pictured this sporulation were evidently led into error by their preconceived notion, and mistook a degenerative process of disintegration for sporulation; this was the case with Canalis, for instance, who, as Bignami notes, described a sporulation in which, to judge from his own designs, the nucleus took no part at all.

"We ourselves, basing our belief upon numerous and careful observations, have always held that the crescents do not multiply in human blood. This affirmation of ours, of which recent experimentation has shown the truth, was based (1) upon the fact that even under the best conditions of research we never succeeded in finding a fission form of crescents, which could with certainty be held to be a sporulation; and (2) upon the fact demonstrated by the researches of Bignami and Bastianelli, that the relapses of the fever are not in relation to the development of the crescent bodies."²

It should be carefully noted, also, that designs of malarial parasites, and interpretations of these, based upon the imperfect methods of staining before the introduction of the Romanowsky method and its modifications, cannot be accepted unless confirmed by this method. For this reason alone the observations of Canalis and those who published confirmatory reports which they have not verified by subsequent research cannot be accepted.³

Of more interest are the comparatively recent reports of Maurer and Neeb, who also claim to have witnessed parthenogenesis of the female crescent. As the reports of these observers are readily accessible, I shall not go into any detail in describing their claims.

¹ *Malaria, Twentieth Century Practice*, New York, 1901, 19, p. 48.

² This criticism, in part, applies also to various observations on the parthenogenesis of tertian macrogametes.

³ For instance, it was long believed that there was a stage in the cycle of the tertian and quartan parasites in which the nucleus could not be demonstrated, because it did not stain by the methods then in use.

Maurer gives two drawings only, which show this so-called segmentation. He himself is not certain of these, for in the description of one of them he places a question mark after the word "gamete." His "Teilungsfigur eines Gameten(?)" cannot in any way, in so far as the picture is a criterion, be differentiated from ordinary asexual segmentation, while the representation of beginning segmentation in the *male gamete* ("männlicher(?) Gamet") will certainly not convince the experienced observer that such was the case.

Neeb found the forms which he describes in one case only, "after two and a half years of fruitless research." Before publishing his report, he sought the advice of the experienced authorities of the German and French Schools of Tropical Medicine. Neeb, with a fairness somewhat rare among those who have published accounts of parthenogenesis, states in detail the objections against his interpretation. As I shall presently give Bignami's criticism of Neeb's views, I will state here only my own opinion. Two of his figures resemble presegmentation, though somewhat atypical, of asexual parasites, as much as they resemble parthenogenesis. The third resembles in some respects a sporulating crescent, and in others beginning reduction, such as is sometimes met with if the slides are not immediately dried. Unfortunately, the plate is in black and white, and not colored. Moreover, a careful comparison of Neeb's drawings with Maurer's demonstrates that the representation by the latter of various phases of the adult asexual form is almost identical with the former's pictures and description. (Compare Maurer's figures 17, 18, and 19 with Neeb's plate.)

Bignami, in a lengthy and minute analysis of the claims of Schaudinn, Karrewij, and Merz and Blüml for parthenogenesis of the tertian gamete, and of Neeb for that of the female crescent, concludes that even if parthenogenesis did take place, which he doubts, the fact by no means implies that it had anything to do with the genesis of relapse. Bignami points out that in the first place Karrewij saw, in one case only, forms similar to those described by Schaudinn. In the second place the patient already had had several attacks of fever, when that one occurred

unexpectedly, at the beginning of which Karrewij observed parthenogenetic forms. Bignami¹ states:

"Now, it is evident that, to explain the cause of relapse, we should expect to find the said forms at the beginning of the first attack of the relapse itself, while everything tends to the belief that the following febrile attacks in the relapse are produced, as a rule, by the regular multiplication of the forms pertaining to the pyrogenous cycle. Therefore the observation of Karrewij is not important concerning the origin of relapse, in the strict sense of the word, but would also lead to the belief that in the ordinary sequence of attacks the gametes might reproduce themselves either entirely, or in company with other forms of the parasites."

Again, according to Bignami, Merz and Blüml found not only the supposed parthenogenetic reproduction, but at the same time common asexual sporulation.² These authors observed very heavy infections, in which the sporulating asexual forms were more than usually abundant. Moreover, in two of these cases there was doubt, as the authors admitted, as to whether they were dealing with relapse or reinfection. Bignami³ objects to the interpretation of parthenogenesis in these cases:

"Now, to diagnose macrogametes in division, and to distinguish such from the numerous schizonts that were found in division at the same time in the preparations, the authors (Merz and Blüml) relied on the description given by Schaudinn and admitted, without further proof, that the distinctive characters of macrogametes in division had been previously ascertained and definitely postulated."

Concerning Neeb's views, Bignami⁴ says:

"The observations of H. M. Neeb are even less conclusive than those that I have mentioned. He refers in his work to having seen in tertian malaria some forms similar to those described by Schaudinn, and adds drawings and a description of three forms of parasites which he interprets as crescents in parthenogenesis. He found these in two preparations from a patient ill with tropical fever, but does not state how long the fever had lasted. It is true, however, that the opinions expressed by various observers who examined Neeb's specimens were not in agreement as to two of the forms; although Prowazek interpreted them as parthenogenetic forms, Le Dantec showed the similarity of these same forms to the ordinary schizogony of estivo-autumnal parasites, and was reserved in his judgment. . . .

"As for the third form in question, Neeb himself admits that he cannot give a precise opinion."

I am of the opinion that a careful evaluation of the above data will not permit of a statement that parthenogenesis is an established phenomenon in the life-cycle of the malarial parasite, or

¹ *Op. cit.*

² Deaderick states this also.

³ *Op. cit.*

⁴ *Op. cit.*

that it is above all the explanation of the etiology of relapse. As Bignami notes, the confirmation is entirely unsatisfactory, and is based on unconnected and isolated observations. To which I venture to add that relapse is one of the common phenomena of malarial infection, and that the cause, when found, will be associated with very many malarial infections, and not with uncommon and seldom observed features of the life-cycle of the parasites.

There are, however, greater objections to attributing the cause of relapse to parthenogenesis of macrogametes than those I have cited. While there may be morphological grounds for the correctness of Schaudinn's hypothesis, biologically the hypothesis is at fault. It is commonly believed today that the gametes are individual parasites endowed *per se* with extraordinary power of resistance against quinine and the protective forces of the body, so that they persist in greater or less number for a considerable time. But the recent work of Thomson¹ in the Liverpool School of Tropical Medicine has demonstrated the error of this belief. The gametes, as individuals, do not persist, but it is an asexual cycle, which produces the gametes, that persists. Thomson showed that gamete formation depends on a function of the asexual generation, and he demonstrated very plainly that the individual gametes do not live very long in the peripheral circulation. So that when gametes persist day after day, in constant number, it is certain that an asexual cycle is simultaneously pursuing its development also, perhaps in the internal circulation.

Ross and Thomson,² moreover, have shown that such a cycle does renew its vitality, if not properly treated, without any visible necessity of parthenogenesis.

Also, it has been possible for me to demonstrate in a series of slides from autopsy cases that, in untreated malaria, all ages of crescent formation may be present, but in proportion to the amount of quinine given and the time in which it has been exhibited, young crescents were found in lesser ratio to adults, until after a week or so; provided there was no organic or other disease to interfere with proper absorption of the drug, only adult crescents, and no

¹ *Ann. Trop. Med. and Parasit.*, 1911, 5, p. 57.

² *Ibid.*

asexual forms were found. Young crescents were always associated in these slides with an asexual generation, showing that the production of the former is a function of the latter. Together, the work of Thomson on the crescents in the peripheral blood, and that of mine on their relation to the asexual cycle at autopsy, show that when the asexual generation is killed, the persistence of crescents stops within two weeks, generally in 10 days. This indicates, as Thomson has pointed out, that the life of the individual crescent is about 10 days, and if these organisms persist after that time, it is because the asexual cycle has not been killed.

These data confirm the old idea that cases which show the gametes for a long time during apyrexia are likely to relapse; but not because of any power inherent in the gametes themselves. The relapse is due, as I have stated, to a renewal of vitality on the part of the asexual generation that produces the crescents.

The hypothesis of Craig is open to many of the objections which have prevented that of Schaudinn from obtaining general acceptance. The actual conjugation of the young parasites has not been reported except by Mannaberg and Craig himself. Perhaps this is because to see the process requires more time and patience than many workers care to give. Henson¹ states: "I myself have observed the phenomena, if such it may be called, in both the tertian and estivo-autumnal infections," but does not state whether he observed it in fresh or stained blood. There is no doubt but that the forms described by Craig, in estivo-autumnal malaria, do occur. While I have never been able to witness actual conjugation in this infection, I have seen all the forms described by him as resulting from it.

Three years ago, while working with a very heavy estivo-autumnal infection, I noticed in the peripheral blood several parasites which did not correspond to the typical organisms of the sexual and asexual cycles. These parasites filled the erythrocytes containing them, stained somewhat like a crescent, but were round, not oval or semi-lunar in shape. They contained scattered pigment, and two to five well defined masses of chromatin. All stages of the asexual generation were present in considerable number in the serial slides taken over 48 hours, and it was very evident that the atypical parasites did not belong to any phase of the presegmenting *Plasmodia*. There were many well defined points of differentiation. The pigment was scattered and rod-shaped, not collected; the chromatin a much lighter red, and not so dense; and the cytoplasm

¹ *South. Med. Jour.*, 1911, 4, p. 130.

of a decidedly different tint. It was suggested that they might be quartans, but no other form of the quartan was found, and moreover, subsequent research showed that they were not of this species.

I did not see these parasites in the peripheral blood again for two years, when in one of the heaviest infections I have hitherto witnessed, I found them in great numbers. The patient was a little Negro baby, only three weeks old. No quinine had been given, and the baby died shortly after admission. In the peripheral blood were all ages of crescent formation and of the asexual cycle and many of the forms referred to, while the smears from the spleen at autopsy showed in very great number more different stages of the life-cycle than I had seen in any other case.

Slides were sent to Dr. Craig, who stated that the atypical parasites were identical with those described by him as resulting from conjugation, and he also very kindly gave me permission to quote him.

My own study of this case has convinced me that the forms mentioned were atypical developing crescents. Except for the distribution of the chromatin in many, and the shape, the other characteristics were the same. All stages of transition between these forms and crescents were observed. I have observed these same parasites at autopsy in several other cases, but always typical crescents were present also, and when both were sufficiently abundant, transitional stages were seen.

It is very evident from this that Dr. Craig has described correctly the forms which he interprets as resulting from conjugation in estivo-autumnal malaria, and others who have seen these slides agree that these forms were present in them. But neither in the spleen nor in the peripheral blood were many doubly infected erythrocytes, and I could not make out any difference between young gametes, except when these had the crescent shape, and young parasites of the "conjugation" type. Moreover, although all stages of sporulation of the asexual cycle were plainly visible, nothing resembling sporulation, except the scattered masses of chromatin, were seen in the "conjugation" forms.

If the form of parasite responsible for relapse is due to conjugation, then certainly fevers which relapse the more often should be those which have the greatest proportion of doubly infected erythrocytes. Now, it is well known that as a rule multiple infection of the erythrocytes is in direct proportion to the severity of the infection. But it is equally well known that the severity of the infection is no indication of relapse. The mildest quartan will

relapse time after time, and one may, as I have done, search in vain in such an infection for a doubly infected cell. As to this, it is Dr. Craig's opinion that it is possible to examine only an exceedingly small portion of the blood, and that doubly infected cells may be so scarce as to escape observation, although plentiful enough to furnish sufficient parasites to cause a relapse. This view has the merit of analogy, for one does not find parasites immediately after the bite of the infected mosquito, and those who believe that relapse is caused by a renewal of vitality in the asexual cycle admit that this cycle may at times be so few in number as to be unobserved in the peripheral blood.

If, however, relapse is due to any latent forms of parasite other than small numbers of the asexual cycle, such forms should be found sometimes in a careful search at autopsy in a country where latent malaria is known to exist in a considerable proportion of the population. Also, the peripheral blood should show these forms at times, when there are no clinical symptoms of malaria.

THE SECOND HYPOTHESIS.

Marchiafava and Bignami,¹ in their excellent treatise on malarial infection, called attention to the fact that relapses at short intervals could best be explained by assuming that a certain number of the parasites survived quinine treatment and the action of the protective forces of the body. This view, however, did not meet with general acceptance, because it was very generally thought that the asexual cycle, when its vitality was impaired, rapidly perished, for very often careful search in the peripheral blood between relapses failed to reveal parasites.

In 1910, Ross and Thomson² published the results of the first research wherein mathematical methods were used to count accurately the degree of infection. By use of the "thick film" method, they were able to show that although the usual method of blood examination might, and very frequently did fail to show parasites in the apyretic interval between relapses, in the thick films the organisms could be demonstrated in some cases during the entire interval. They also found that relapses so produced

¹ *Op. cit.*, p. 362.

² *Ibid.*

had no relation whatever to gametes or "resistant forms," but depended entirely on the degree to which the asexual parasites resisted quinine or the protective forces of the body. They¹ state:

"Moreover, the general trend of the curves suggests that they [parasites] were not found on these days [days in the middle of the apyretic period] only because their numbers were a little too few for detection. The parasite curve, at its height during a pyrexial period, generally falls very rapidly at first and more slowly later, and tends to reach its lowest about half-way between the two apyrexial periods. At this point it may or may not remain above the detectable limit [by thick film methods]. After this it was observed [by Thomson], especially in Cases 7, 17, 23, and 24, to begin mounting, slowly at first, until, when it reached the pyrogenic limit, another pyrexial period commenced. All this is scarcely compatible with the speculation that the apyrexial periods are due to the abrupt death of most of the asexual *Plasmodia*, or to their conversion into 'resting stages' etc. Nor do such speculations appear to be at all necessary. It is easy to see that the survival of comparatively small numbers of the asexual forms will suffice to keep the infection alive, not only for the short periods observed by us, but for 'relapses of long interval,' and for months and years."

These conclusions of Ross and Thomson, based as they are on accurate research of the highest degree, have been accepted, even by those who advocate some special form of the parasite as responsible for long interval relapses, as explaining relapses at short intervals. They are not accepted by Craig, Deaderick, and Henson as explaining long interval relapse.

In 1910, Bignami² published the result of his research for many years previously into the pathogenesis of relapse. He takes up in great detail the hypothesis of parthenogenesis, but unfortunately does not discuss that of intracorpuseular conjugation. In this paper, which ranks with those of Ross and Thomson in recent importance, after a careful examination of the claims made for a "resting stage" of the parasite, he concludes that there is no basis for such claims. He abandons, provisionally, his former hypothesis, by which he explained long interval relapses as due to merozoites which in some manner became invested with a protective coating, because after years of examination of autopsy specimens he could find no confirmation of his view. It is significant to note here that he, also, did not find any other form that could be interpreted as a "resting stage."

Bignami then explains relapse as due to the persistence of the asexual cycle, arguing from the viewpoint of analogy and biology.

¹ *Ibid.*

² *Op. cit.*

He believes that the asexual parasites become *immune* either to the effect of quinine, or to the protective forces of the body. This immunity may be complete or only partial. He cites as analogous to this view the recent experiments of Ehrlich¹ with trypanosomes. These showed that trypanosomes may be made immune against the protective forces of the body, against certain drugs, and against combinations of certain drugs, but that a strain immune against some is not immune against others. Experiments similar to these with other protozoa are also noted. He then cites the well-known immunity of malarial parasites in some instances to quinine. He concludes that this immunity, whether against quinine or against the protective forces of the body, will explain any case of relapse, whether at long or short intervals.

Three years ago, while working on the relation of the fever curve in malaria to the life-cycle of the parasites, I observed 50 cases in which quinine was not exhibited for some time. In a few of these only was it necessary to give the drug to control the fever, which disappeared spontaneously in most cases with rest in bed. It was noted then that many of the patients had parasites in the peripheral blood during apyrexia, and that an increase in the number of these always foretold a relapse. It was also observed that gametes were not always present, but when they were, there was no alteration in their morphology. The asexual parasites, however, underwent marked changes, and this without treatment. This fact led me to believe that the asexual cycle, having once lost its vitality, could not renew it, and I thought that a "resting stage" of some kind would be necessary before the infection could renew itself. Later, in studying quartan malaria, I thought that I had found such a stage.² Subsequent research, however, has convinced me that I was wrong, and that the forms which I took to be "latent parasites" were atypical stages of the life-cycle.

Not only did I observe ordinary relapse while studying the cases above mentioned, but I saw several cases of relapse with a species of parasite which replaced the one present when the patient was admitted. These cases were very carefully studied. The first infection, whether estivo-autumnal or tertian, was entirely replaced by the second infection. As one group increased in number, the other decreased. At this time Schaudinn's work on parthenogenesis, and Craig's conjugation hypothesis were known to me. I thought that since it was possible for me to observe relapse from the time when the parasites first appeared in the blood, and with a species different from the original infection, so that there could be no question of a renewal of vitality on the part of the latter, it would not be difficult to attribute the cause to one or the other hypothesis. However, I could find no verification of either, only asexual parasites were observed early in the secondary infections, and gametes of these did not appear until later. Neither did the original infections always disappear as gametes or "resistant forms," but often as disappearance of the asexual cycle. This fact was

¹ *Chemotherapeutische Trypanosomen-Studien*, Leipzig.

² *Proc. Canal Zone Med. Soc.*, 1910, 3.

particularly noted. Of course, some of the cases showed gametes after the disappearance of the asexual forms, but this was not a constant feature.

Having in mind Marchiafava and Bignami's description of double tertian and double and triple quartan cases that relapse in the original form, I sought for these also. In this I was successful, and observed from the time of admission, through an apyretic period in which the parasites disappeared (I was using the thin film method), relapse in two cases of triple quartan and one of double quartan, several of double tertian, and one of mixed estivo-autumnal and quartan. All of these relapsed in the form of the first observed infection; the triple quartan as triple quartan, the double quartan as double quartan, etc.

The mixed estivo-autumnal and quartan case is of particular interest. The patient was a little Columbian boy, about seven years old, who had come from Cartegena to Colon about a year before his admission to this hospital. He was admitted to the service of Dr. A. B. Herrick, chief of Surgical Clinic, for Hirschsprung's disease. Pending operation, he was placed in my ward. His blood examination on admission was negative for malarial parasites. About a week after admission, he had a chill, and manifested a rise in temperature to about 103. The blood examination showed a moderate single quartan infection. On the following day a heavy estivo-autumnal infection was also seen, and in proportion as this increased the quartan infection disappeared, so that two days later only a few quartan gametes were seen. Quinine was given, and the fever promptly stopped. Two weeks later the operation was successfully performed. About seven months later, the patient having in the meantime moved to a different locality, he returned for further observation. While in the ward he again developed fever, with quartan parasites, which were followed two days later by estivo-autumnal organisms.

The mother of this boy, a woman of exceptional intelligence for her class, is employed as a school mistress by the Panama government. She told me that before coming to Panama, her son had repeatedly suffered from quartan fever, and she described very accurately the quartan symptoms, and called them by name. It would appear, then, that the first quartan attack was a relapse. The estivo-autumnal attack may or may not have been primary. The second attacks of the two, following in the same sequence as at first, were very likely due to a relapse of each species, as quartan malaria is very rare indeed relatively in Panama, and the patient had not lived in the same locality as before the first attack.

I have also, for the past two years, sought especially for adult conjugation and parthenogenetic forms in the peripheral blood. These are very rarely encountered, and only as I have described above. They are almost always in relation to relapse or reinfection, as they are found, as I stated, only in anemic blood, and there was a history of previous malaria in all cases but one, that of the three-weeks-old baby.

Finally, in an effort to find the form of parasite latent in the internal circulation, I examined smears from over 200 autopsies, 150 of which were consecutive. Several times I found parasites when none had been encountered in the peripheral blood, and the cause of death was other than malaria. There were also cases of death from traumatism, in which smears from the spleen and rib marrow showed parasites. This work will be reported later in full, but I can state at present that at no time did I find any parasites other than forms of the sexual or asexual cycles, and these in about equal numbers. Nothing that I myself would call a "resting form" was seen, except the parasites that Dr. Craig interprets as conjugation forms, and that I think are atypical crescents.

III.

In presenting the foregoing account of the various hypotheses as to the etiology of relapse, although I have stated my own opinions of their validity, I have tried to give impartially the findings of various workers. It is now my purpose to examine these hypotheses particularly with reference to their biological significance, in order to ascertain what relation they bear to observed facts in the life-history of the *Plasmodia*.

These facts I have stated previously. They are, briefly:

1. Relapse is a common feature of malarial infections.
2. Relapses follow most often, in the order named: untreated primary infections; insufficiently treated infections; and infections treated late in their course. Although rarely, they follow the most vigorously treated primary infections. And it is easier to effect a cure in uncomplicated than in complicated infections.

From these data it is evident that the cause of relapse is intimately connected with two factors in the life-cycle of the *Plasmodia*: (a) a stage when quinine as usually given does not eradicate the infection; and (b) this stage is in intimate relation to the time the infection has persisted. Any hypothesis that does not accord with these factors cannot be accepted unless it be admitted that the factors are false. Also, a hypothesis should agree with the clinical observations in relapse which so many careful workers have verified.

If it be admitted that parthenogenesis actually happens, it by no means follows that this is the cause of relapse. The evidence adduced shows that in all reported cases of the phenomenon except that of Schaudinn, and this is doubtful, an active asexual cycle was persisting at the same time, and further, that the parthenogenesis occurred during the progress of the relapse, and not at the beginning. It is necessary, then, to prove beyond question that there has been no confusion of parthenogenetic forms with atypical segmentation. The latter is known to occur, while the former, it may be said in all fairness, are somewhat arbitrarily classified, as Bignami¹ has pointed out. Ross² has very carefully reviewed the evidence brought forward by Schaudinn, and states:

"A patient who had long suffered from *P. vivax* (the tertian parasite) was attacked on the 29th of April and the 1st of May, and was found to contain both sporoids (asexual parasites) and gametids (gametes). A rally (period of intermission between relapses) now occurred, during which daily examination disclosed *varying* numbers of gametids only. On the 25th of May these parasites were *more plentiful*. Next day curious changes were noted in the female gametids, suggesting that they were producing

¹ *Op. cit.*

² *The Prevention of Malaria*, New York, 1910, p. 112.

spores similar to those ordinarily produced by the asexual sporoids. The author [Schaudinn] considered this to have occurred independently of fertilization by the male gametids, and to be due to parthenogenesis. The same day (26th of May) the patient had a slight rise of temperature to 38.4°C ., and in the evening ordinary young sporoids were found. Next day *only* these forms occurred. On the 28th of May there was a typical attack with temperature reaching 40.75°C ., and with the usual sporoids. The author carefully described the parthenogenetic forms, and traced the corresponding changes in the nucleus. . . .

"I note especially that between the 1st and 25th of May the number of gametids *varied*, and was increased on the latter date. But this variation suggests that they were being produced all the time. The gametids are supposed to be produced from ordinary spores; so that we are forced to infer that a number of sporoids, some of them generating gametes, were present in the patient's body, although they were too few to be detected in the small quantities of blood examined by the author. On the 25th of May they probably increased in number sufficiently to produce a slight attack of fever, and then were mistaken for parthenogenetic gametes. On the 28th of May they produced a typical attack; and that is all. The supposed nuclear changes were reported on evidence of no great value. The cells were not actually observed undergoing the development which the author describes. He merely inferred the existence of the development from a study of different cells in what he thought were different stages of that development."

Now, in tertian malaria, very often the entire cycle of development of the gametes can be followed in the peripheral blood, and is completed in from four to five days. Continued persistence of the gametes for longer than this period, as Ross notes, is a sure indication of the persistence also of an asexual cycle. Adult tertian gametes do not persist as individuals for 26 days. For if quinine be exhibited in full doses, the gametes, no matter how plentiful at first, will disappear in four or five days. I have demonstrated this by many experiments, working both with thin and thick films. If the gametes persist longer than this period, it means that the quinine is not being properly absorbed, and it should be given hypodermically or intravenously; either method, when properly administered (of which more later) will cause prompt disappearance. Nor does the fact that in Schaudinn's case the gametes disappeared after the sporulation of May 28th prove that such disappearance was due to their sporulation. In untreated tertian malaria, after the asexual parasites have disappeared for a while from the peripheral blood through the action of the agencies which bring about the so-called spontaneous cure, it is not at all unusual to observe a similar disappearance of the gametes a short time later.

In view of these facts, and our recent knowledge that when the thick film method is used the asexual parasites do not disappear all at once in untreated malaria as in the case reported by Schaudinn, not only is it impossible to exclude the presence of an asexual cycle at the time when the clinical symptoms of relapse were manifested, but the probability is that such a cycle was present, as Ross notes.

Relapse is a common feature of malarial infections. Mannaberg¹ notes that "malaria is one of those infectious diseases in which a relapse may be considered an essential feature," and goes on to state that "it makes little difference whether the first disease (attack) recovered spontaneously or was cured by the action of quinine." In passing, it may be observed that Mannaberg's opinion does not speak well for the methods of treatment used against malaria at the time when he wrote. Now, to explain the rarity with which parthenogenetic and "latent" types are observed, when the result of their further development is so common, it is assumed by those who postulate the necessity for such types of the malarial parasites that in the resting stage these types remain in the internal circulation, and further, that in most instances their sporulation takes place in the internal circulation, so that neither the types nor their further development are often seen in the peripheral blood.

But there are several other types of the *Plasmodia* that are rarely found in the peripheral blood; particularly certain phases of the estivo-autumnal parasites—the young gametes, and segmenting and presegmenting forms. Yet these are often to be found in abundance in the spleen and bone marrow at autopsy, after death from pernicious malaria, and in malarious countries are not infrequent at autopsy when death has occurred from a cause not at all connected with malarial infection. Craig reports seven cases of the latter—three of tertian, and four of estivo-autumnal infection—and notes that he did not find gametes. Bignami² also reports such cases, and I have observed quite a number myself. Dr. S. T. Darling and Dr. H. C. Clark, in their extensive autopsy experience here, inform me that this occurrence is

¹ *Op. cit.*, p. 340.

² *Op. cit.*

not at all infrequent. However, none of the authorities mentioned, nor have I myself, found at autopsy in acute or latent malaria any type of parasite other than the normal forms of the life-cycle according to our interpretation, except the forms previously referred to, that Craig holds to be the result of conjugation, and I believe to be developing crescents. It may be argued, as has been noted, that parthenogenetic and "latent" forms are too few to be observed when the infection is latent, or that they had all sporulated, so that only the normal forms of the life-cycle were observed in the instances referred to above. But such a supposition is based upon speculation, and not upon actual observation, and, until supported by positive data, may not be accepted.

Parthenogenesis will not explain why triple quartan and double tertian infections relapse as such. Although many cases of malaria relapse at definite intervals, such as 7, 14, or 21 days, many more relapse at irregular intervals, as is very well shown by Craig¹ in his careful study, and no definite period of latency can be predicted. If it is difficult to explain the mechanism by which any latent infection is awakened, it is much more so to infer that gametes and "latent" forms renew an infection by sporulation at intervals of 24 hours, and so start again the cycles of triple and double infections.

It is admitted that if the continued persistence of gametes were due to an inherent longevity of these as individuals, parthenogenesis as a factor in the etiology of relapse could be accepted. But a definite life-cycle for gametes has been demonstrated. They originate in the asexual cycle, and their continued persistence over a period longer than 10 days or two weeks in estivo-autumnal malaria means a continued persistence of the asexual cycle that produces them. The cycle of the tertian gametes is, as I have noted, from four to five days. I have also worked out the cycle of the gametes in quartan malaria.² It takes place in six days, after which, in properly treated cases, or in cases where there is a spontaneous disappearance of the asexual forms, the gametes rapidly disappear.

¹ *The Malarial Fevers*, New York, 1909, p. 161.

² *Op. cit.*

As has been stated, the gametes are not definite factors in relapse. This, as is well known, occurs quite independently of the production of sexual parasites, whether in the peripheral blood or in the internal circulation, as far as the observation of many authorities and my own work is a criterion. If the gametes did not have a definite life-cycle, but persisted indefinitely, independently of the fate of the asexual cycle, if parthenogenesis were the etiological cause, since it is well known that adult gametes apparently are not affected by quinine, then treated cases in which the gametes are the latest forms of the parasites observed should always relapse. But many cases of primary and relapsing infections, in which the gametes were the last forms seen, have been permanently cured by prolonged and vigorous treatment with quinine, and in a time well within the limit of observed periods of relapse. The observations of Ross and Thomson¹ on relapsing cases in Liverpool, and many on primary and relapsing cases in this hospital, have shown that under proper treatment the crescents and other gametes will disappear within the limits of the life-cycles assigned to them.

If these facts are true, there can be no doubt but that the hypothesis of parthenogenesis does not accord with the factors common to relapse, which have been noted previously. While the older gametes appear to be immune against quinine, there is no proof that they persist as individuals for the long periods of latency that are recorded, some of them for over two years, nor, as has been noted, is the formation of gametes in relation to the observed factors in the etiology of relapse.

Many of those who uphold the single observation of Schaudinn, and who regard it, as Bignami states, "as though it were a datum of fact at this time unassailable" would do well to consider the criticism of Ross and of Bignami on this point. They would do well, also, to remember that the supposed confirmations of Schaudinn's work, when carefully analyzed, refer much more to a process that took place during the course of a relapse, rather than to a process that occurred at the beginning of one.

¹ *Ann. Trop. Med. and Parasitol.*, 1910, 4, p. 137.

The conjugation hypothesis of Craig¹ is founded on far more sufficient data and observation than that of Schaudinn. Here is a problem of interpretation of process and resultant forms, and a concordance of these with known clinical and biological factors in relapse, rather than morphological description of a doubtful sporulation of gametes at a time when a normal asexual cycle must have been in progress. Craig's study covered a period of nearly seven years, and he states that he observed conjugation in about 600 cases. He claims for the process that it results in the union of the nuclei of two young, unpigmented parasites, with subsequent reduction of chromatin, and a union of cytoplasm as well. He states that conjugation "occurs only after the plasmodia have multiplied in the usual manner for several generations," and that it is observed only in those malarial infections which have persisted for some time, adding that it never "occurs during the first few days of a malarial infection," and further, "I have never observed the process in cases in which but two or three paroxysms had occurred prior to treatment, but after the infection has persisted for a week or more, intracorpuseular conjugation is invariably observed." Neither does the phenomenon exhibit itself when quinine has been administered in sufficient doses promptly on the appearance of the symptoms of the primary or of the recurrent attacks. It is especially frequent when parasites are abundant, and there are many doubly infected cells, but may be found in any infection that has persisted for some time, if the parasites are abundant enough to allow of extended observations.

It has been argued against Craig's hypothesis that he has mistaken double infection of the erythrocyte in the early period of the asexual cycle for conjugation. In my experience, this criticism is not altogether well founded. The larger forms, described by Craig as the result of conjugation, certainly bear no resemblance, in my opinion, to doubly infected cells. His interpretation of the appearances in stained specimens, described by him as the beginning of conjugation, is, however, a matter for argument. And in differing with him in some respects as to this interpretation, as I shall do, it is well to remember that his experi-

¹ *Jour. Infect. Dis.*, 1910, 7, p. 285.

ence with all aspects of malaria and the *Plasmodia* is that of one who has made a careful study for many years.

As far as I can ascertain, no one has hitherto attempted to confirm or to disprove Craig's hypothesis by repeating his observations to any extent and for a sufficient time. Such confirmation or denial as has come to my notice has been based rather on a priori reasoning than on careful study. On this account I shall state only the conclusions which I have reached as the result of three years' study on this hypothesis. It is proper, I believe, to mention here that Dr. Craig, in a recent personal communication, states that he is of the opinion that more than one factor in the cycle of the parasites enters into the etiology of relapse, and while he believes that short term relapses are explained by assuming a continuation of the asexual cycle, he does not agree that such an assumption will explain relapses at long intervals.

In my study, I have never seen actual conjugation of living parasites. This does not, however, in my opinion preclude the possibility of such a process. Conjugation, according to Craig, by no means follows every double infection of an erythrocyte, and is a phenomenon that requires several hours for its completion; so that the difficulty of observing the entire occurrence is obvious, when it is remembered that two parasites may lie in apposition for an hour or more, and then separate. By the time one has made several such observations, the cycle has passed the stage when conjugation takes place. Craig¹ and Mannaberg,² however, state positively that they have witnessed the entire phenomenon, and their descriptions are very convincing.

In stained specimens I have repeatedly seen many of the forms described by Craig as various stages during conjugation. But when I attempted to find these in the fresh specimen immediately afterward, I was not successful, as I observed only different aspects of multiple infection of the erythrocytes. It is a matter of common occurrence to see in stained specimens of any heavy estivo-autumnal infection the forms described as the immediate result of conjugation, and similar forms can be seen in a control fresh specimen, as far as

¹ *Op. cit.*, p. 301.

² *Ibid.*, p. 300. See also "Malarial Diseases," Nothnagel's *Encyclopedia of Practical Medicine*, Philadelphia, 1905, p. 52.

the appearance of the parasite is a criterion. Forms with two "vacuoles" separated by a band of cytoplasm, and with a common periphery, I have repeatedly observed under the conditions when conjugation should take place, but after being watched for a while, such forms always returned to the original ring shape, and, indeed, not infrequently reverted to the first-described condition.

In untreated estivo-autumnal infections I have never observed any but normal forms of the asexual cycle. Only in tertian and quartan parasites have I seen the atypical segmentation mentioned. I have followed in the peripheral blood more than once a complete cycle of the estivo-autumnal parasites, in the beginning of which there was, in stained specimens, every appearance, as far as I could judge, of conjugation; but the later phases of the cycle showed only forms which I interpreted as normal, nor were there atypical delayed parasites, such as one would have expected had conjugation occurred. As to the adult forms mentioned previously, I interpret them as rather atypical developing crescents, and these Dr. Craig interprets as the result of conjugation.

Further, at no time in my study of tertian and quartan infections could I demonstrate any similitude of conjugation. This was seen by me only in estivo-autumnal infections. From the account given by Craig of the large tertian parasites resulting from conjugation, and from the appearance of these in the photographs he presents, I feel sure that such parasites are the same as those I have described as resulting from atypical segmentation, and similar to the parthenogenetic forms of Schaudinn, especially Craig's Fig. 9, which he himself thinks is similar to a so-called parthenogenetic form, as he¹ states: "This is the form which may have been considered by Schaudinn as a macrogamete undergoing parthenogenesis." It is very true that Craig's pictures are, as he claims, easily differentiated from normal segmenting and presegmenting parasites, but whether they are different from the ones which I hold to be the result of atypical development is something that can be determined only by other workers.

I have seen very many such forms toward the close of tertian and quartan cycles, and particularly in the latter instance, when a

¹ *Jour. Infect. Dis.*, 1910, 7, p. 311.

very careful study of the earlier stages had shown but few, if any, doubly infected cells, or forms resembling the phases of conjugation. For this reason especially I believe that the forms in question are atypical, and not the result of conjugation. In tertian and quartan infections such parasites are also seen at times when the rest of the cycle is advanced or delayed, thus lending color to Craig's belief that conjugating forms do not accompany the rest of the cycle in development; but since double infection is so common in these infections in the Canal Zone, it would not be possible to say that these parasites did not belong to a second cycle.

From the viewpoint of morphology, I am convinced that the forms as described by Craig in stained specimens for the actual conjugation and further development of malarial parasites do exist. The difference of opinion as to the precise classification of these forms is, however, a matter of interpretation, and as such I leave it.

The relation of these forms to the known factors in relapse should be carefully investigated. If they occur, as Craig states (and in this respect my findings corroborate his), only after the infection has continued for some time, then the hypothesis offers an explanation of the relapses that so often follow infections not treated until the clinical symptoms have been well established. Unfortunately, Craig could not follow the complete cycle of his forms, and those that he describes as resembling segmentation were not found at the beginning of relapse, but later in the infection, after conjugation had been established.

And here I would again take exception to the acceptance of sporulation which is claimed to result from parasites other than those of the asexual cycle, when such sporulation is found during the febrile stage, or at the close of it. If any latent form of the parasite, whether a gamete or the result of conjugation, which does not pertain to the asexual cycle, by its sporulation brings about a relapse, such sporulation should be observed at the beginning of the renewed cycle of the brood of parasites that is responsible for relapse, and not at the height of activity or at the close of such a cycle. Any sporulation observed under the latter conditions is a phenomenon that can be interpreted only as happening during

ordinary schizogony, and bears no relation to the etiology of relapse, as Bignami well observes, no matter how rare or peculiar the form in question. Unless such "latent" forms are so plentiful that one period of sporulation would determine the symptoms of relapse, in which case certainly the process should be observed in the peripheral blood, the parasites must increase through several generations before symptoms are evident, and such parasites as are seen during the pyrexia, or at the close of it, are of necessity products of those whose awakening from latency caused the relapse. Yet various authors who describe the sporulation of "latent" forms find these, not at the beginning of the schizogony that ultimately results in the clinical manifestations of relapse, but during the period of such schizogony, or at its close, and claim that such parasites are those which initiate the relapse. Such reasoning describes effect for cause. Nor does it aid the argument to assume that when the forms are found at the end of the relapse they are those which will continue the infection, for there are no facts to prove this assumption but only conjectures.

The increase of parasites resulting ultimately in the clinical manifestations of relapse, from the time these appear in the peripheral blood to the period of greatest number and through decline, has been carefully studied by Ross and Thomson¹ in a number of cases, and I have done the same. We have found nothing other than the normal sporogony and schizogony during this increase and decline (except the atypical forms as noted), nor have we found any sporulation of "latent" forms at the beginning, or development of these at the end. If it be argued that the "latent" forms, at the beginning of renewed vitality, are in the internal circulation, or are so few in number as to escape notice, then again the hypothesis rests upon conjecture, and not upon established fact. For careful examination of autopsy preparations when latent malaria has been concurrent with the cause of death, and when parasites were not found in the peripheral blood, even after very careful examinations, has failed to demonstrate any form of *Plasmodia* other than those I hold to pertain to the sexual and asexual cycles.

¹ *Op. cit.*

Conjugation, which is admitted to occur only late in the infection, will not explain many cases of relapse following only one paroxysm, nor relapses following infections in which the process was not observed. While some authors hold that the more severe the infection, and the longer it persists without treatment, the more likely is relapse to follow, I do not agree altogether with this belief. Certainly, clinical observations agree that untreated infections which persist over a long time are very prone to relapse, but very slight tertian infections often relapse after only one or two mild paroxysms, when the parasites have been very scanty indeed, and the quartan infection is notorious for its relapses, without regard to the severity of the infection and the number of parasites, or the duration of the disease.

However, it must be admitted by any impartial student of malaria that the hypothesis of conjugation itself, whatever may be the interpretation of the process, rests on the careful and extended observations of an experienced authority, and can be confirmed or denied only by a study as careful as his.

IV.

In the introduction to this paper I gave as my definition of relapse:

"A return of the fever and parasites after the former has ceased and the latter have disappeared from the peripheral blood, reinfection being excluded. I do not include those cases in which quinine has not been administered in doses sufficient to clear the peripheral blood of parasites. Such cases are not true relapses, for the original infections are only diminished."

So widespread is the belief that there are two kinds of relapses—one due to a simple renewal of vitality at short intervals, without further change of the asexual generation; and the other, to a renewal of vitality at long intervals of some "resistant" or "latent" form, derived from the asexual cycle—that in order to keep clearly in mind the various hypotheses as to the etiology of relapse I have not as yet modified my original definition. The result of my studies for the past three years, however, has convinced me that there is but one primary cause of relapse, a renewal of vitality on the part of the asexual cycle, and that this is true, whether relapse

takes place a week or a year after the original infection has subsided. From this viewpoint a relapse is not an occurrence apart from the original infection, but merely a phenomenon manifested at different times by an infection that has not been eradicated, in so far as the symptoms of chill and fever are concerned, and as such I wish to consider it in the remainder of this paper.

The relation between the numerical quantity of the infection with its toxic products, and the reaction of the human organism as manifested by pyrexial symptoms, varies so greatly that the amount of infection which produces definite febrile attacks in one individual may show itself in another as a non-febrile cachexia, and so be overlooked. Clinically, then, while a relapse may be defined as a return of the febrile symptoms due to an increase in the number of parasites, biologically it is the response of the asexual cycle to a renewal of vitality by which the number of organisms again increases, and as such may appear as a return of fever, as cachexia, as neuritis, or as one or more of the many diverse symptoms associated with what is called "chronic malaria." This latter term, it may be noted, means no more than that the primary infection has not been eradicated. The wave-like rise and fall in number and activity, with lower and higher levels that remain stationary over varying periods, is a phenomenon common to the cycles of many protozoa, free living as well as pathogenic, and the latter has been particularly well shown by Ross and Thomson¹ in their study of a case of human trypanosomiasis.

As long as the malarial infection persists, one may expect at times some clinical manifestation of it, and what is commonly termed a relapse—recurrent pyrexial symptoms at varying intervals after the primary manifestations have subsided—is, as noted, no more than one of the many responses of the human organism to the products of an increased number of parasites following the renewal of vitality in the asexual forms residual after the primary attack, or after each succeeding pyrexia.

The hypothesis that the asexual generation alone, and without alteration other than increase and decrease in its reproductive power, is responsible for relapse as I have defined it above, is upheld

¹ *Ann. Trop. Med. and Parasitol.*, 1911, 4, p. 261.

recently by Bignami and by Ross and Thomson, and the reasons for their belief have been given elsewhere in this paper. To these I shall now add the conclusions to which I have come as the result of my own study.

To substantiate the hypothesis that the asexual generation is the only phase in the life-cycle of the *Plasmodia* which is the etiological factor in relapse, it is necessary to assume, as far as can be done by the proof at hand, two propositions:

1. That the asexual cycle is endowed with a potential of vitality which enables it to persist, at times in numbers too small to be detected in the peripheral blood, over a period of at least two years, corresponding to the longest known interval between a primary infection and a relapse, or between one relapse and another.

It may be noted here that much of the data as to the exact length of time between these phases of the malarial infection is very inaccurate, and extremely unsatisfactory. Anyone who has worked to any extent on the diagnosis of malaria will refuse to accept as positive an account of a malarial infection which is not satisfactorily verified by accurate blood findings, no matter how definite the clinical symptoms. Nor will an account of a relapse¹ after such an infection be accepted, unless it is shown beyond doubt that there was no chance of reinfection in the meantime; that parasites similar to those of the primary infection were found during the relapse; and that the individual who reports the relapse knows exactly what a malarial parasite looks like, whether in fresh blood or stained. These conditions are made necessary by the reports in recent literature which are not verified by sufficient data, and I am sure that no one who has worked for several years with fresh blood and stained, and with good stains and bad, will object to these restrictions. Under them, the longest period of relapse that I have been able to ascertain—two years—was reported some years ago by Marchiafava and Bignami.

2. That the asexual cycle can become relatively immune to the protective forces of the body, and also to the action of quinine.

1. The assumption that the asexual cycle can persist over long periods, although in very small numbers, is one that many authorities on malaria refuse to accept; and, indeed, it is not altogether susceptible of proof by direct methods. However, very many facts in support of this assumption can be legitimately adduced. It may be admitted, at the outset, that the same arguments which militate against an acceptance of the view that the asexual cycle renews itself by parthenogenesis, or by sporulation of "latent" forms when these are too few to be detected in the peripheral blood

¹ In this note I use the term "relapse" in the sense of the original definition.

or elsewhere, would also hold against the belief that this cycle renews itself from asexual forms which are also too few to be detected, were it not for the additional proof that can be brought forward, by analogy as well as by fact.

Analogy, as Bignami observes, while not of value unless supported by correlative data, takes on importance when such data are available.

The life-cycle of the *Plasmodia* in man may be compared to those of other pathogenic protozoa which also pass in man only one part of their existence. The best known of these are the *Leishmaniae*, the *Trypanosomata*, and the *Entamoebae*. *T. pallidum* as far as I know, goes through its entire life-cycle in man; so that the only pathogenic protozoa (if indeed they are protozoa), in which there is an extra-corporeal cycle, that show in the human organism "latent" or "resting" phases, are the spirilla forms responsible for relapsing fever, and in these there is no certainty as to such phases.

Now Ross and Thomson have shown by their precise methods that the continuance of trypanosome infection in the human body is due only to a persistence of the asexual phase. I am also informed by Dr. S. T. Darling,¹ who has made a careful study of the animal trypanosomiasis which he has found in the Canal Zone, that he has observed in infected animals none of the so-called "latent" bodies or "resistant" forms of the parasites. Similarly, I have failed to find any positive account of a "resting" stage, or a "latent" form of either *L. tropica* or *L. donovani* in the human body. In fact, one of the highest authorities on these species, Leonard Rogers,² states that only one phase is so found, that of the cycle of the non-flagellate bodies. Yet sleeping sickness, oriental sore, and kala-azar persist over long periods, and the infections are maintained, as far as I know, solely by the persistence of organisms that correspond to the asexual cycle of *Plasmodia*.

Amoebic dysentery is a protozoon infection well known for its tendency to "relapse" over many years. Like *Plasmodia*, *Entamoebae* produce forms, the cysts, apart from the asexual cycle,

¹*Jour. Infect. Dis.*, 1911, 8, p. 467.

²*Fevers in the Tropics*, London, 1908, p. 85.

and these have an extra-corporeal existence, which is intended for the transfer of the infection to another host, although unlike in *Plasmodia*, these forms do not have to pass through another organism. Whether autogamy takes place in these cysts, so that they are self-fertilized before renewing the infection elsewhere, or whether there is in the intestinal canal conjugation of the young *Entamoebae* that Darling¹ has shown to be derived from these cysts, is disputed; but it is a fact that as far as is known at present, infection with *Entamoebae* can and does persist in man for many years, without any known cause for continuation other than the life of the asexual cycle of these parasites. There are, I am aware, observations on a supposed conjugation of the vegetative forms, but these have not been confirmed, nor do those who made them insist on their validity.

Free living protozoa, as shown by the experiments of Calkins² and of Woodruff,³ when the environment is favorable, can reproduce themselves for many generations without necessity for parthenogenesis or for conjugation. Woodruff, repeating Calkins's original experiments, has carried *Paramecium* through an asexual cycle of over 3,000 generations.

These facts will serve to show that, in so far as analogy has value, there is no reason why the asexual cycle of the *Plasmodia* should not persist for a time equal to the longest recorded interval between a primary infection and a "relapse," or between one "relapse" and the succeeding one. I might add here again, for emphasis, that in the other pathogenic protozoa, as in *Plasmodia*, what has not infrequently been taken for a "latent" form has later been recognized as merely a product of degeneration.

To come now to more direct proof of the vitality of the asexual cycle of the malarial parasites, it can be shown that these do persist at times in sufficient quantity to be demonstrated in the peripheral blood, for apyretic periods that are equal to those which many observers claim to be too long for the persistence of this cycle. I myself have seen a quartan infection, that apparently neither

¹ *Arch. Int. Med.*, 1913, 11, p. 1.

² *Op. cit.*

³ *Jour Am. Med. Assn.*, 1912, 59, p. 1724.

increased nor decreased in quantity, show itself daily in the peripheral blood for a period of six weeks, during which there was a considerable period of apyrexia. It might have run on in this way much longer had it not been killed by large doses of quinine. Others, particularly Marchiafava and Bignami, have reported similar cases, which lasted in a like manner even longer. As for persistent negative findings in the peripheral blood, in the interval between febrile relapses, such as Thayer¹ in his excellent monograph puts such stress upon, it should be remembered that the "thin film" method, or else the examination of fresh blood, was used, and neither method can compare in such instances with the accuracy of the "thick film" method originated by Sir Ronald Ross.²

Nor do I think it is logical to assert, in view of the data which I shall now present, that the asexual cycle perishes in the intervals when even by the "thick film" method no parasites can be found in the peripheral blood. The finding of parasites in the peripheral blood means no more than that infection has attained to a certain numerical quantity, as Ross has pointed out.³ If the infection maintains itself in this quantity for six or eight weeks, what reason is there to suppose that it cannot maintain itself in smaller numbers for the same, or even a longer period? The finding of the parasites in the peripheral blood is an indication of a high potential of vitality; there are no grounds for supposing that a lower potential may not be in force without such manifestation.

That the infection may persist in small numbers is, I believe, sufficiently clear, when one considers the course of what is called "chronic malaria." Marchiafava and Bignami make a distinction of value with reference to that term.

On the one hand, are the cases in which the fever is mild, and the pyrexial attacks are infrequent. The only symptoms of such cases are those referable to the acute attacks. On the other, are cases in which the febrile relapses may be mild or severe, and occurring with more or less frequency, but in which appear the well-

¹ *Lectures on the Malarial Fevers*, New York, 1901, p. 184.

² *Op. cit.*, p. 91; also *South. Med. Jour.*, 1911, 4, p. 688 and p. 725.

³ *Op. cit.*, p. 115.

known symptoms of chronic malaria—*anemia*, enlargement of the spleen and liver, edema, palpitation, and dropsy.

Now, the symptoms of this second group are not brought about solely by the effects of four or five febrile attacks, unless these are prolonged and of unusual severity. For many persons who, by reason of reinfection or relapse have had a similar number of febrile attacks, when these have been treated promptly and energetically, show no further disturbance than that associated with the attacks themselves. The other symptoms often develop in the apyretic intervals between the febrile manifestations, even when the latter are mild, and are due to an additional factor. This factor, even before the hypothesis that the asexual cycle persists between febrile relapses was advocated, Mannaberg looked on as “an obstinate persistence of the virus”; and Marchiafava and Bignami defined chronic malaria: “When the infection of the organism continues for months and even years.” It is clear from a study of their work that these authorities recognized a persistence of the infection, but at that time they did not correlate this persistence with that which occasions relapses after long apyretic intervals.

But the variation in intensity of malarial infection is one of degree only, and such infection continuing over a long period of time does not of necessity always produce pyrexia, or even other symptoms.

In the medical service of Ancon Hospital, Dr. W. E. Deeks years ago recognized post-malarial symptoms such as *anemia*, neuritis, cephalgia, and minor complaints occurring at more or less frequent intervals after the primary infection, or between relapses, when pyrexia was absent. And he found that such cases often do not improve on quinine by mouth, but clear up rapidly after two or three hypodermic injections of quinine.

There is also a group of cases similar to these, but further advanced, in which the *anemia* is more profound, and gastrointestinal disturbances and other signs of toxemia are more apparent. These cases also may resist quinine by the mouth, during apyretic intervals, yet the condition yields rapidly to hypodermic or intravenous administration. Similar cases are not uncommon,

with and without the finding of parasites in the peripheral blood. Through the courtesy of Dr. D. M. Molloy, of the Philippine General Hospital, I can present the details of a very instructive case:¹

The case was that of an American civil engineer, aged 23, who contracted an estivo-autumnal infection while in the Philippines, in July, 1911. He was treated in the hospital with quinine by mouth until the infection was controlled. He left the hospital in good condition, *but still had a few crescents in his peripheral circulation.*

In September he returned to the hospital, "all run down," as he expressed it, but had had no paroxysms during the interval, and had no fever at the time of this admission. The patient was almost cachectic, there was marked anemia, and again the blood examination showed *a few crescents*. This time he was given daily doses of hydrochlorid of quinine hypodermically, 1 gram, later supplemented by 1.6 grams by the mouth, and also iron and arsenic tonic. Improvement was slow, and the patient remained in hospital for two weeks. No parasites were found on discharge.

In December he returned to hospital in a worse condition than before, although in the meantime he had had no recurrence of the febrile attacks, and had consistently taken the anti-malarial tonic prescribed. Blood examination *still showed crescents*. The patient was so weak that he could not perform his duties, and had lost much flesh. Some edema of the extremities had developed, and there was present a marked secondary anemia.

He was now given 1.3 grams of the bihydrochlorid of quinine and urea intravenously each day for three days; then 2 grams every other day for a week; and then the latter dose every third day for another week. The drug was given in 200 c.c. of normal salt solution.

The improvement in his condition, Dr. Molloy notes, was so rapid as to be almost marvelous. No crescents were found after the third day, and the patient gained 16 pounds during the three weeks that he was in hospital. Subsequent blood examinations were made monthly until July, 1912, and parasites were not found again, while the patient has remained in good health.

Only to the persistence of an asexual cycle from July to December could the symptoms be attributed, because no one claims that the gametes themselves have any ill effect on the human body.

Between the mildest cases of chronic malaria and those I have just cited, and between the latter and the severest cases with enlargement of the spleen and liver, profound anemia, edema, and other indications of great organic disturbance, are all grades of transition, so that no sharp distinction can be made. And such cases, as noted, occur with and without the finding of parasites in the peripheral blood, and with and without subjective pyrexia

¹ The following case is cited almost verbatim from the notes sent by Dr. Molloy, and I wish to take this opportunity to thank him for the report.

at intervals. Probably a carefully kept temperature record would show varying degrees of pyrexia at some time in all such cases, as Mannaberg, and Marchiafava and Bignami believe. Yet any of these symptoms, except those due to lesions beyond repair, will yield to proper and continued quinine treatment. And since the clinical manifestations of "the obstinate persistence of the virus" are so varied in degree, it is a logical inference that the "virus" itself also varies in quantity and virulence. For by no hypothesis of "resting stages" or of "latent" parasites can be explained the continuation of the symptoms during the apyrexial periods, with prompt disappearance after a suitable administration of quinine. Only the persistence of an asexual cycle of the *Plasmodia*, with the formation of toxin and direct and indirect destruction of the erythrocytes, will explain such a clinical picture. I believe that there is abundant evidence in the above data to demonstrate that it is possible for the asexual cycle to persist over several months without giving rise to subjective febrile symptoms, even when the parasites are not found in the peripheral blood.

Further, it by no means follows that the asexual cycle of the *Plasmodia* is absent from the internal circulation because it cannot be found in the peripheral blood after repeated and careful examination. More than once, in diseases such as abscess of the liver, or septicemia, at the onset or on admission to hospital it has seemed as though malaria were a factor, although careful examinations of the blood have been made here repeatedly with negative findings. Yet at autopsy forms of the asexual cycle were found with more or less frequency in smears from the spleen and marrow. Also, in cancer, chronic nephritis, and similar diseases of long duration, when the patients were in hospital several months under observation without any symptoms of malaria and without any findings of parasites in the peripheral blood, we have found these at autopsy in the spleen and marrow, in forms of the asexual cycle.

As I have previously noted, I do not believe that these findings are the result of sporulation of gametes or of "resting" stages. In cases of latent malaria I have never found at autopsy either gametes or what might be called "resting" stages of tertian or quartan infections, and if such forms were present, it is very

difficult to understand why they should have been repeatedly overlooked.

2. The demonstration that the malarial parasites of the asexual cycle may become immune to the protective forces of the body is self-evident from a consideration of any untreated case of malaria in which they persist for a week or more, or indeed from the fact that they persist at all, and need not be discussed further.

The effect of quinine on the *Plasmodia* is, however, so intimately related to complex factors in absorption, as to make difficult or impossible at this time a definite statement as to the precise degree of immunity that the parasites may acquire. Direct observation shows there must be some immunity so acquired. Ross and Thomson¹ describe a remarkable case of double infection with tertian and estivo-autumnal parasites which plainly shows such immunity.

The patient was admitted on the 23d of October, after having been ill at intervals for 159 days, during which time he had taken quinine thrice daily for a month, and at other intervals as well. On admission he had fever, and a double infection with gametes and asexual forms of *P. falciparum*, and with asexual forms of *P. vivax*, was found. Quinine hydrobromid in liquid form, in doses of 10 grains three times a day, was given, and continued for 17 days, during which period the temperature remained normal. No asexual parasites were found after five days.

From the 3d until the 9th of November, the blood was not examined. On the 10th there was a rise in temperature, the blood was examined, and no parasites were found. The quinine was discontinued. The temperature of a true malarial type, persisted, however, and on the 14th another blood examination was made, this time showing parasites of both species, and gametes of *P. falciparum* also began to appear. From the 14th to the 18th of November quinine was given in doses of 20 grains per day. However, the fever continued, and the parasites increased in number from a few on the 14th to 18,000 per cubic millimeter on the 21st. On the 22d after the dosage had been increased to 30 grains per day, an extra dose of 30 grains was given intramuscularly, and 12 grains of methylene blue in pill form was given also daily. In three days no asexual parasites could be found, but the crescents persisted for 14 days longer or about three days after their normal life-cycle, which would have been expected.

It was supposed that perhaps the quinine might not have been properly absorbed, but a urinalysis made by Dr. G. C. Simpson showed that such was not the case, for the patient was excreting 13 grains a day out of the 30 administered, which, in the author's opinion, was the usual amount.

Bignami² reports a similar case of Torti's and Angelini's, when after a gram of quinine had been given by hypodermic

¹ *Ann. Trop. Med and Parasitol.*, 1912, 5, p. 539.

² *Op. cit.*

daily for a month, a relapse, with the finding of parasites in the blood, followed almost immediately. Dr. Molloy's case also shows an acquired immunity against quinine on the part of some of the asexual parasites.

Cases very similar to these have been observed here not infrequently. Dr. Molloy states that he has seen relapse even after intravenous injections. I have observed one such case in which it was possible to say that the attack was a relapse. As far as I can determine, cases that relapse after intravenous doses of quinine, are those in which small doses have been given, or that have been treated with quinine by mouth previously. Mannaberg¹ is of the opinion that it is difficult to attack organisms in the parenchyma of the spleen and marrow with the drug, and I think that there are very good reasons for this belief.

For, in cases dying from malaria after three or four days of vigorous treatment, very often it is only in the spleen and marrow that parasites can be found. Several times at autopsy I have seen a large number of parasites in the placenta, after three or four days' treatment, when the peripheral blood was negative after prolonged search, and when but few parasites were found in the spleen and marrow. Smears of the spleen and marrow contain relatively a much smaller number of erythrocytes (in which the asexual cycle lives) than do smears of the peripheral blood, so that when parasites are found without trouble in the former when the latter is negative, it is obvious that in the former, for some reason, the parasites have not been affected as in the latter. In fact, in the spleen and marrow not a few of the parasites appear normal, although most of them show the effects of the quinine.

I have also noted, in following at autopsy cases that have died of malaria after three to five days' treatment, that parasites, though perhaps absent from the peripheral blood at death, are found most frequently in the spleen and marrow after oral administration of quinine, less frequently after treatment by hypodermic injections,² and with least frequency after three or four doses of 22.5 grains intravenously. It used to be not at all uncommon to find large

¹ *Op. cit.*, p. 344.

² These are given in a dilution of 1:20 of normal salt solution, as first originated by Dr. W. E. Deeks.

numbers of parasites after three or four days' treatment with quinine by mouth, but if quinine has been given intravenously for two or three days in doses of 22.5 to 30 grains, no matter how heavy the infection was on admission, the parasites are always scanty in the spleen and marrow at autopsy, and not infrequently only a very few are found.

I have found no reason to believe that only quinine immune parasites seek the circulation of the spleen and marrow. I hold that such parasites as are found there are those which by reason of this locality have escaped the full effects of the drug. These, and other considerations presented elsewhere in this paper, have led me to present the following hypothesis as an explanation of the fact that certain asexual parasites survive the action of quinine, and later take up a cycle of increased reproduction.

In common with others who have worked on malaria, I believe that the circulation of the bone marrow and spleen is the favored location of the *Plasmodia* in the human host. In latent malaria, when the infection is very scanty, the bone marrow and spleen are the only places where parasites are found, notwithstanding, as I have noted, the relative infrequency of erythrocytes in smears as compared with smears of the peripheral blood, and here, in small numbers, the parasites go through a normal asexual cycle. Here also, in estivo-autumnal malaria is where the gametes are formed, particularly in the bone marrow, as Marchiafava and Bignami first noted. When the parasites have multiplied beyond a certain extent they find their way into the peripheral blood, though what may be the conditions that cause this increase as manifested in febrile relapse I cannot explain.

By reason of this location, the parasites that inhabit it are to a less extent affected by quinine than those in the peripheral blood, as is shown by the persistence of the former when none can be found in the external circulation. Sufficient quinine given by the mouth, except in very severe cases, will rapidly kill the parasites in the peripheral blood, but does not always get rid of those in the internal circulation, as the persistence of gametes after the time of their cycle of development demonstrates. (See Dr. Molloy's case, and others reported in any textbook on malaria.) These residual para-

sites, since they are only partly affected by quinine (probably some are not at all affected), acquire an immunity against the drug similar to that acquired by other protozoon parasites of man under like conditions. This immunity, however, seems to pertain particularly to the parasites of the spleen and marrow. As long as there is a supply of quinine in the peripheral blood, those in it are killed. It is very unusual to observe a relapse with parasites in the external circulation during efficient quinine treatment. When such occurs, there has been faulty absorption of the drug, because the parasites disappear rapidly after subsequent hypodermic or intravenous medication. Nor does the withdrawal of quinine necessitate an immediate response by the parasites to such an extent that an increase follows, which may be detected in the peripheral blood. The asexual parasites may not increase in number for long periods, or they may increase only sufficiently to give rise to symptoms of toxemia, as was shown in the consideration of chronic malaria.

It is not possible at present to determine the conditions that control the wave-like increase and decrease which take place until the infection has been eradicated or dies spontaneously. But that such increase and decrease occur has been shown, and likewise the fact that instead of periods of increase and decrease there are also periods when the number of parasites is stationary over some time, as in the case of quartan malaria cited. And, as Sir Ronald Ross has well demonstrated, it is illogical to infer that the asexual cycle cannot outlive those periods in which it is found in the peripheral blood. The entire data of chronic malaria, as noted, tell against such an inference.

Provided there is no fault of absorption in the stomach and the intestinal canal, I do not know why it is that so often quinine by the mouth is less effective than by hypodermic injection, but I do know that this is true. Dr. W. E. Deeks has suggested that, since quinine by mouth, before reaching the systemic circulation, must pass through the liver, possibly some change in its toxicity against the *Plasmodia* takes place. It is quite certain, as Ross and Thomson's case well demonstrates, that proof of retention of quinine by urinalysis is no indication of whether the drug is killing the parasites in the internal circulation. Also cinchonism, which many rely

upon as an indication of successful exhibition of the drug, is unreliable as a test of toxicity against the parasites, for cinchonism is more indicative of the patient's susceptibility to quinine than it is that the remedy is doing the work for which it was given.

The fact that of all systems of medication the intravenous method in high dilution, as first recommended by MacGilchrist,¹ is the most successful in freeing the circulation of the spleen and marrow of parasites, suggests that even in the process of absorption after hypodermic injections some of the effects of the drug are lost.

SUMMARY.

If the hypothesis be accepted that the asexual cycle alone is the cause of relapse, and under certain conditions takes on a relative immunity against quinine, an explanation is offered of all the factors concerned in the etiology of relapse, and I know of no other hypothesis which will explain satisfactorily the correlation between these factors and the data which to this time have been collected about relapse. I may summarize these factors, which were given early in this paper, in relation to the asexual cycle as follows:

1. Relapse is one of the most common factors in malarial infection; and certainly the asexual cycle is that phase of the malarial parasites found most frequently associated with the primary infection and relapse, and with one relapse and the succeeding one.

2. Relapse follows frequently the so-called spontaneous cure of malaria, because the asexual cycle in such an instance often persists, in numbers that can be detected by the "thick film" method, or slightly below this limit, in the intervals of apyrexia.

3. Infections treated insufficiently with small doses of quinine will in all probability relapse, because the parasites of the asexual cycle in the spleen and marrow are very slightly, if at all, affected thereby.

4. Relapse is less likely to occur when the infection is promptly and vigorously treated, because the older the asexual cycle, the more resistant to quinine it becomes, as shown by numerous clinical observations.

¹ *Quinine and Its Salts*, Cited in *Paludism*, Simla, 1911.

5. When a relapse occurs, with manifestation of parasites in the peripheral blood, during the administration of quinine by mouth in sufficient doses, faulty absorption of the drug is sure to exist, and the method of medication should be changed.

6. The asexual generation does not have an unlimited potential of vitality. That is why, if death does not supervene in the course of a malarial infection, the infection itself will in time die out, but often not until it has done irreparable damage.

7. It is easier to eradicate an infection in persons in good health, because in these the natural protective forces of the body aid the action of quinine.

The hypothesis also concurs with the two factors in the life-cycle of the parasites, which are intimately connected with the etiology of relapse: because (*a*) quinine given by the mouth very often does not eradicate the asexual cycle in the marrow and spleen, and the residual parasites become immune; and (*b*) the longer the asexual cycle persists, the easier it acquires immunity against the drug.

The practical importance of the hypothesis lies in the value it has as a guide to treatment of malaria. Small doses of quinine, even in the mildest infections, serve only to render the asexual cycle relatively immune, so that larger doses, which, if they had been given early in the attack might have eradicated the parasites, are later without effect.

We have found this to be true by our clinical experience here in Ancon Hospital. In the treatment of malaria in our American employees, it was observed by Dr. Deeks that when the liquid preparation of quinine sulfate which we use was given in doses of 20 grains per day by mouth, while acute attacks were often controlled, recurrent cases in large numbers followed at very short intervals later, and in the treatment of these, larger doses were necessary. A routine treatment of 20 grains on diagnosis, and 10 grains three times a day later, for at least 10 days, was then established, and this we used for several years. This noticeably diminished the number of recurrent cases (we can determine relapses positively in individual cases, only under a few conditions, such as recurrence while in hospital) among the Americans, but not suffi-

ciently among the more poorly nourished European laborers. Dr. Deeks then instituted a treatment of 45 grains per day, in doses of 15 grains each. This method has practically eradicated recurrent malaria among the Americans, and to a large extent among the European laborers. These latter, however, treat mild infections themselves with small doses of quinine, and so produce a relatively quinine immune asexual cycle that is more difficult to eradicate. They are, moreover, heavily infected with syphilis, a condition which, as noted, tends to lessen the efficacy of the usual treatment until the syphilis itself is under control.

It should be stated that in the past six years our malaria rate has fallen to about one-fifth of the maximum, so that reinfections are proportionately less infrequent, but the rate was never relatively very high among the Americans except in 1905 and 1906, so that later in this class, recurrences at short intervals, which were plentiful enough, were nearly always due to relapse.

It will, of course, be obvious to anyone who is familiar with the recent work of Ross and Thomson, and of Bignami, on the etiology of relapse in malaria, that the hypothesis I have brought forward is a combination of their views, and is not altogether original with me. I desire to take this opportunity to acknowledge my indebtedness to these authorities for the use I have made of the material they have furnished me.

I wish also to thank those authorities throughout the world who sent answers to the letter of inquiry on this subject, which was addressed to them by the Department of Sanitation of the Isthmian Canal Commission. In the complete report on this subject, which will be made later by Dr. Deeks and myself, full acknowledgment of all the information received will be made.

I am indebted to many of the physicians on the medical side of Ancon Hospital for the opportunity to study cases as they occurred, particularly to Dr. Roland Connor for furnishing me with material to study quartan cases.

And I wish to thank Dr. S. T. Darling, chief of Board of Health laboratory here, and Dr. H. C. Clark, pathologist, for furnishing me with material to study from autopsy cases.

Also I thank Col. John L. Phillips, Medical Corps, U.S. Army, acting chief sanitary officer, for permission to publish this paper.

EXPLANATION OF PLATE I.

These figures are intended to show, according to my interpretation, some of the phases of the *Plasmodia* which are held by others to be various stages in parthenogenesis or in conjugation.

They are drawn to the scale of 7μ , or the diameter of a normal erythrocyte, to one-fourth of an inch. The measurements were taken with a Leitz Filar micrometer,

after computing its value by use of an object micrometer. I found this method more satisfactory than the use of a camera lucida. The magnification is about 800.

FIG. 1.—Multiple infection of an erythrocyte with three young estivo-autumnal parasites. Note the overlapping of two of the parasites.

FIGS. 1a-32.—These were taken from a quartan infection that had persisted for six weeks under observation. There was a hospital record of two previous admissions for quartan infection within the previous eight months. There was marked anemia at the time when the drawings were made.

FIGS. 1a-7, 11, 12, 23, 24.—Normal schizogony of the quartan parasite. The other figures show various atypical developmental and segmentation phases. Figs. 9 and 10 particularly show part of the chromatin undivided, the so-called *Restkörper*, with the rest in collected masses prior to segmentation. All phases between this and normal segmentation may be followed in the figures. Fig. 19 shows a very large quartan parasite in irregular division, as compared with Fig. 7. In Figs. 25-32 inclusive the segmentation is very atypical.

FIGS. 33-38.—Conjugation in stained specimens, as described by Craig. Note the division of the chromatin, and the extrusion of part of it in Fig. 36.

FIGS. 39-45.—Drawn from the same specimen. These show various phases from the youngest trophozoite to beginning presegmentation. It can be seen that the nuclear phenomena, as represented by division and arrangement of the chromatin, are the same in these single parasites as in those represented as conjugating.

FIGS. 46-48.—These show double infection of the cell, because the parasites are nearly 24 hours old, as can be told by the formation of the "signets," while conjugation takes place only between very young parasites. Yet at the junction of the parasites, there is no line of demarkation, which is also true in conjugating forms.

In the infection from which Figs. 33-48 were taken, every phase of normal schizogony could be followed in the peripheral blood at the same time. Notwithstanding the large number of parasites arranged as in Figs. 33-38 inclusive (conjugation forms) all stages of further development to segmentation were typical, even in many of the doubly infected cells; in some of which latter two fully developed segmenting forms could easily be made out, while others contained two parasites at different stages of growth.

Parasites very similar to those in Figs. 9-32 inclusive are pictured by W. S. Harrison for the tertian parasite, in the *Journal of the Royal Army Medical Corps*, London, Vol. 13, No. 6 (December, 1909). In a forthcoming publication by Dr. David Thomson and myself, Dr. Thomson has drawn the phases of crescent formation referred to in the preceding article, and these will show the difference between the asexual cycle and the variations in type in the sexual cycle.



